# REPORT

## ASSESSMENT OF CONTACT HYPERSENSITIVITY TO

[redacted]

## IN THE ALBINO GUINEA PIG

## (MAXIMISATION-TEST)

**NOTOX Project 338704**
**NOTOX Substance 111834/B**

## CONFIDENTIALITY STATEMENT

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STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

*The OECD Principles of Good Laboratory Practice* which are essentially in conformity with:

United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

Japanese Ministry of Agriculture, Forestry and Fisheries. 59 NohSan, Notifications No. 3850.

Japanese Ministry of Economy, Trade and Industry. Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85.

Japanese Ministry of Health, Labor and Welfare. Ordinance No.21.

Study Director:
Drs. F.M. van Otterdijk

Date: 25 April 2002

Management:
W.J.A.M. Frieling DVM

Date: 26 April 2002

## QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

| DATES OF QAU INSPECTIONS/AUDITS | REPORTING DATES |
|---|---|
| on-site inspection(s) (Process) | |
| 04-06 February 2002 | 22 February 2002 (Animal Unit) |
| protocol inspection(s) (Study) | |
| 08 November 2001 | 08 November 2001 |
| report audit(s) (Study) | |
| 11+16 April 2002 | 16 April 2002 |

Head of Quality Assurance: C.J. Mitchell B.Sc.

Date: 26-4-02

## SUMMARY

Assessment for Contact Hypersensitivity to [redacted]n the Albino Guinea Pig (Maximisation Test).

The study was carried out based on the guidelines described in: EC Commission Directive 96/54/EC, Part B.6, "Skin Sensitisation", OECD No. 406, "Skin Sensitisation" and EPA OPPTS 870.2600 "Skin Sensitisation", August 1998 and based on the method described by Magnusson and Kligman, "Allergic Contact Dermatitis in the Guinea Pig - Identification of Contact Allergens".

Test substance concentrations selected for the main study were based on the results of a preliminary study.
In the main study, ten experimental animals were intradermally injected with a 1% concentration and epidermally exposed to a 5% concentration. Five control animals were similarly treated, but with vehicle alone (corn oil).
Two weeks after the epidermal application all animals were challenged with a 2% test substance concentration and the vehicle. A second challenge was performed one week later with a 2% test substance concentration and the vehicle.

In the first challenge phase skin reactions of grade 1, were observed in three experimental animals in response to the 2% test substance concentration. No skin reactions were evident in the control animals. Scaliness was seen in some treated skin sites of among the experimental animals.

To confirm the results of the first challenge, a second challenge was performed one week later. In the second challenge phase skin reactions varying between grades 1 and 2, were observed in seven experimental animals in response to the 2% test substance concentration. No skin reactions were evident in the control animals. Scaliness was seen in some treated skin sites among the experimental animals.

Since scaliness was not observed in control animals, it may be indicative of sensitisation in the experimental animals. Therefore, scaliness was taken into account for the establishment of the sensitisation rate.
The skin reactions observed in the first challenge phase in response to a 2% test substance concentration in six (of the ten) experimental animals were confirmed in the second challenge phase and were therefore considered indicative of sensitisation, based on the absence of any response in the control animals.
These results indicate a sensitisation rate of 60 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), [redacted]should be labelled as: may cause sensitisation by skin contact (R 43).

## PREFACE

| | |
|---|---|
| Sponsor | [redacted] |
| Study Monitor | Dr. C.L.J. Braun<br>SHERA, Regulatory Affairs |
| Testing Facility | NOTOX B.V.<br>Hambakenwetering 7<br>5231 DD 's-Hertogenbosch<br>The Netherlands |
| Study Director | Drs. F.M. van Otterdijk |
| Study Plan | Start : 11 February 2002<br>End : 28 March 2002 |

## TEST SUBSTANCE

The sponsor is responsible for all test substance data unless determined by NOTOX.

| | |
|---|---|
| Identification<br>Chemical name<br>CAS RN | [redacted] |
| Description | Clear colourless liquid |
| Batch | 1510-14 |
| Purity | See Certificate of Analysis |
| Test substance storage | In refrigerator in the dark |
| Stability under storage conditions | Stable |
| Expiry date | 01 January 2003 |
| Density | Approx. 1160 $kg.m^{-3}$ |
| Stability in vehicle | |
| • Corn oil | Unknown |

## TEST SUBSTANCE PREPARATION

| | |
|---|---|
| Vehicle | Corn oil |
| Rationale | The vehicle was selected based on a pretest performed at NOTOX. |
| Preparation | The test substance formulations (w/w) were prepared within 4 hours prior to each treatment. No adjustment was made for specific gravity of vehicle. Homogeneity was obtained to visually acceptable levels. |

## PURPOSE AND RATIONALE

The purpose of this study was to evaluate whether the test substance induces contact hypersensitivity in guinea pigs after intradermal and epidermal exposure of the animals under the conditions described in this report.
This study should provide a rational basis for risk assessment in man.
The Maximisation test is selected because it is regarded as the most sensitive and the preferred method with regard to testing for sensitisation potential.

## GUIDELINES

As required by the Dutch Act on Animal Experimentation, the study protocol was reviewed and agreed by the Article 14-functionary and the Ethical Committee of NOTOX (DEC NOTOX 97-03-11) as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures described in this report were based on the following guidelines and test method:

European Community (EC), Council Directive 67/548/EEC, Annex V, Part B, Methods for the Determination of Toxicity, as last amended by Commission Directive 96/54/EC, Annex IV C, B.6: "Skin sensitisation", Official Journal of the European Communities No. L 248, 1996.

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.406, "Skin Sensitisation", Paris Cedex, 1992.

Environmental Protection Agency (EPA): Health Effects Test Guidelines OPPTS 870.2600. "Skin Sensitisation", August 1998.

"Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens" Magnusson B. Kligman A.M., 1970 published by C.C. Thomas, Springfield, Illinois, USA.

## ARCHIVING

NOTOX B.V. will archive for at least 10 years raw data, protocol, report, all specimens and test substance reference sample. No data will be withdrawn without the sponsor's written consent.

## TEST SYSTEM

| | |
|---|---|
| Species | Himalayan strain, albino guinea pig (SPF-quality)<br>Recognised by international guidelines as the recommended test system (e.g. OECD, EC).<br>Source: Biotechnology & Animal Breeding Division (RCC Ltd.), Füllinsdorf, Switzerland. |
| Number of animals | Experimental group: 10 females.<br>Control group: 5 females.<br>(females were nulliparous and non-pregnant). |
| Age | Young adult animals (approx. 5 weeks old) were selected. |
| Identification | Ear tattoo. |
| Reliability check | The results of a reliability test performed not more than 6 months previously are summarised in the Appendix. Similar procedures were used in the reliability test and in this study. |

## ANIMAL HUSBANDRY

Conditions
A controlled environment was maintained in the room with optimal conditions considered as being approximately 15 air changes per hour, a temperature of 21±3°C, a relative humidity of 30-70% and 12 hours artificial fluorescent light and 12 hours dark per day.
Temporary deviations from the maximum level for relative humidity (with a maximum of 20%) did occur which might have been caused by cleaning procedures in the room. Based on laboratory historical data these deviations were considered not to have affected the study integrity.

Accommodation
Group housing of 5 animals per labelled metal cage with wire-mesh floors. The acclimatisation period was at least 5 days before the start of treatment under laboratory conditions.

Diet
Free access to standard guinea pig diet, including ascorbic acid (1000 mg/kg); (Charles River Breeding and Maintenance Diet for Guinea Pigs, Altromin, Lage, Germany). Certificates of analysis were examined and retained in the NOTOX archives. Hay (B.M.I., Helmond, The Netherlands) was provided twice a week.

Water
Free access to tap water. Certificates of quarterly analysis for tap-water were examined and retained in the NOTOX archives.

PRELIMINARY IRRITATION STUDY

A preliminary irritation study was conducted in order to select test substance concentrations to be used in the main Study. The selection of concentrations was based on the following criteria:
- The concentrations are well-tolerated systemically by the animals.
- For the induction exposures: the highest possible concentration that produced mild to moderate irritation (grades 2 - 3).
- For challenge exposure: the maximum non-irritant concentration.

Series of test substance concentrations were tested. Practical feasibility of administration determined the highest starting-concentration for each route. The starting- and subsequent concentrations were taken from the series: 100% (undiluted), 50%, 20%, 10%, 5%, 2%, 1% and if needed, further lower concentrations using the same steps.
The test system and procedures were identical to those used during the main study, unless otherwise specified. The six animals selected were between 4 and 9 weeks old. No body weights were determined.

Intradermal injections:
A series of four test substance concentrations was used, the highest concentration being the maximum concentration that could technically be injected. Each of two animals received two different concentrations in duplicate (0.1 ml/site) in the clipped scapular region. The injection sites were assessed for irritation 24 and 48 hours after treatment.

Epidermal application:
A series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be applied. Two different concentrations were applied (0.5 ml each) per animal to the clipped flank, using Metalline patches# (2x3 cm) mounted on Medical tape#, which were held in place with Micropore tape# and subsequently Coban elastic bandage#. The initially used animals receiving intradermal injections were treated with the lowest concentrations and two further animals with the highest concentrations. After 24 hours, the dressing was removed and the skin cleaned of residual test substance using water and/or vehicle.
The resulting dermal reactions were assessed for irritation 24 and 48 hours after exposure. Based on the results in the initially treated animals, two additional animals were treated in a similar manner with four lower concentrations at a later stage.

#. Suppliers: Lohmann GmbH, Neuwied, Germany (Metalline) and 3M, St. Paul, Minnesota, U.S.A. (Medical tape, Micropore and Coban).

MAIN STUDY

INDUCTION - Experimental animals

Day 1 The scapular region was clipped and three pairs of intradermal injections (0.1 ml/site) were made in this area as follows:
A) A 1:1 w/w mixture of Freunds' Complete Adjuvant (Difco, Detroit, U.S.A.) with water for injection (Fresenius AG, Bad Homburg, Germany).
B) The test substance at a 1% concentration.
C) A 1:1 w/w mixture of the test substance, at twice the concentration used in (B) and Freunds' Complete Adjuvant.
Note: One of each pair was on each side of the midline and from cranial A) to caudal C).

Day 3 The dermal reactions caused by the intradermal injections were assessed for irritation.

Day 8 The scapular area between the injection sites was clipped and subsequently treated with 0.5 ml of a 5% test substance concentration using a Metalline patch (2x3 cm) mounted on Medical tape, which was held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 48 hours exposure, the skin cleaned of residual test substance using water and the dermal reactions caused by the epidermal exposure were assessed for irritation.

INDUCTION - Control animals

The control animals were treated as described for the experimental animals except that, instead of the test substance, vehicle alone was administered.

CHALLENGE - All animals

Day 22 One flank of all animals was clipped and treated by epidermal application of a 2% test substance concentration and the vehicle (0.1 ml each), using Patch Test Plasters (Curatest®, Lohmann, Almere, The Netherlands). The patches were held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle using water. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing.

Day 29 A re-challenge was conducted approximately one week after the first challenge, to clarify the results in the first challenge. The contralateral flank of all animals was similarly treated.

## OBSERVATIONS

| | |
|---|---|
| Mortality/Viability | Twice daily |
| Toxicity | At least once daily. |
| Body weights | Prior to start and at termination of the study. |
| Skin reactions | Skin reactions were graded according to the following numerical scoring systems. Furthermore, a description of all other (local) effects was recorded.<br>Whenever necessary, the treated skin-areas were clipped at least 3 hours before the next skin reading to facilitate scoring. |

Grading Irritation Reactions*:

Erythema and eschar formation:

No erythema ..... 0
Slight erythema (barely perceptible) ..... 1
Well-defined erythema ..... 2
Moderate erythema ..... 3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) ..... 4

Oedema formation:

No oedema ..... 0
Slight oedema (barely perceptible) ..... 1
Well-defined oedema (edges of area well-defined by definite raising) ..... 2
Moderate oedema (raised approximately 1 millimeter) ..... 3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure) 4

(*. Intradermal reactions were assessed for erythema only or, if necrosis is present, the diameter of necrosis.)

Grading Challenge Reactions:

No visible change ..... 0
Discrete or patchy erythema ..... 1
Moderate and confluent erythema ..... 2
Moderate erythema and swelling ..... 3
Intense erythema and swelling ..... 4

## INTERPRETATION

The results for the experimental animals at the challenge phase were compared with the results for the control animals.
Positive skin reactions (grade 1 or more) will be considered signs of sensitisation provided that such reactions are less severe or are less persistent in the control group.
A sensitisation rate (%) was calculated as follows: the number of sensitised animals as a proportion of the total number of animals in the experimental group.
The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).

## RESULTS

### PRELIMINARY IRRITATION STUDY

The results of the intradermal injections and epidermal exposures for the selection of suitable test substance concentrations for the main study are described in Table 1.

Based on the results, the test substance concentrations selected for the main study were a 1% concentration for the intradermal induction and a 5% concentration for the epidermal induction exposure. A 2% test substance concentration was selected for the challenge phase.

### MAIN STUDY

**Induction phase**
The skin effects caused by the intradermal injections and epidermal exposure during the induction phase are given in Table 2.

**Challenge phase**
First Challenge
Skin reactions of grade 1, were observed in three experimental animals in response to the 2% test substance concentration. No skin reactions were evident in the control animals. Scaliness was seen in some treated skin sites of among the experimental animals (see Table 3).

Second challenge
To confirm the results of the first challenge, a second challenge was performed one week later.

Skin reactions varying between grades 1 and 2, were observed in seven experimental animals in response to the 2% test substance concentration. No skin reactions were evident in the control animals. Scaliness was seen in some treated skin sites among the experimental animals (see Table 4).

**Toxicity / Mortality**
No mortality occurred and no symptoms of systemic toxicity were observed in the animals of the main study.

**Body Weights**
Body weights and body weight gain of experimental animals remained in the same range as controls over the study period (see Table 5).

## CONCLUSION

Since scaliness was not observed in control animals, it may be indicative of sensitisation in the experimental animals. Therefore, scaliness was taken into account for the establishment of the sensitisation rate.
The skin reactions observed in the first challenge phase in response to a 2% test substance concentration in six (of the ten) experimental animals were confirmed in the second challenge phase and were therefore considered indicative of sensitisation, based on the absence of any response in the control animals.
These results indicate a sensitisation rate of 60 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), [redacted] should be labelled as: may cause sensitisation by skin contact (R 43).

TABLE 1: PRELIMINARY IRRITATION STUDY

SKIN REACTIONS AFTER INTRADERMAL INJECTION

| Animal number | Conc % | 24 hours after injection | | 48 hours after injection | |
|---|---|---|---|---|---|
| | | Erythema (grade) | Necrosis (mm) | Erythema (grade) | Necrosis (mm) |
| 795 | 10# | | 10 | | 10 |
| | 5 | | 8 | | 8 |
| 800 | 2 | | 3 | | 4 |
| | 1 | 3 | | 2 | |

#. A 10% test substance concentration was considered the highest concentration that could technically be injected. Higher concentrations did not pass through the injection needle.

SKIN REACTIONS AFTER EPIDERMAL EXPOSURE

| Animal number | Conc. % | 24 hours after exposure | | 48 hours after exposure | |
|---|---|---|---|---|---|
| | | Erythema (grade) | Oedema (grade) | Erythema (grade) | Oedema (grade) |
| 785 | 100 | n | 1 | n | 1 |
| | 50 | n | 1 | n | 1 |
| 790 | 100 | n | 2 | n | 2 |
| | 50 | n | 1 | n | 1 |
| 795 | 20 | n | 0 | n | 0 |
| | 10 | 0 | 0 | n | 0 |
| 800 | 20 | n | 0 | n | 0 |
| | 10 | 1 | 0 | n | 0 |
| 783 | 5 | 1 | 0 | 0 p | 0 |
| | 2 | 0 | 0 | 0 | 0 |
| 784 | 1 | 0 | 0 | 0 | 0 |
| | 0.5 | 0 | 0 | 0 | 0 |

p. Scaliness
n. signs of necrosis

TABLE 2: INDUCTION READINGS

| Animal Number | Intradermal injection (DAY 3) A | | B | | C | | Epidermal exposure (DAY 10) D | |
|---|---|---|---|---|---|---|---|---|
| Control | E | N | E | N | E | N | Erythema | Oedema |
| 831 | 3 | | 2 | | 2 | | 0 | 0 |
| 832 | 2 | | 1 | | 2 | | 0 | 0 |
| 833 | 2 | | 2 | | 2 | | 1 | 0 |
| 834 | 2 | | 1 | | 2 | | 0 | 0 |
| 835 | 2 | | 1 | | 1 | | 0 | 0 |
| Experimental | E | N | E | N | E | N | | |
| 836 | 2 | | 2 | | 2 | | 2 | 0 |
| 837 | 2 | | 2 | | 3 | | 3 | 0 |
| 838 | 3 | | 1 | | 3 | | 3 | 0 |
| 839 | 2 | | 2 | | 3 | | 2 | 0 |
| 840 | 2 | | 2 | | 4 | | 2 | 0 |
| 841 | 2 | | 2 | | 4 | | 2 | 0 |
| 842 | 3 | | 2 | | 3 | | 3 | 0 |
| 843 | 2 | | 2 | | 3 | | 2 | 0 |
| 844 | 2 | | 2 | | 2 | | 2 | 0 |
| 845 | 2 | | 2 | | 3 | | 3 | 0 |

A. 1:1 Mixture of FCA and water for injection.

B. A 1% test substance concentration (Experimental); vehicle (Control).

C. 1:1 Mixture of FCA and a 2% concentration (Experimental) or vehicle (Control).

D. A 5% test substance concentration (Experimental); vehicle (Control).

Skin effects intradermal injections:

E. Erythema (grade)

N. Signs of necrosis (mm in diameter)

TABLE 3: CHALLENGE READINGS, FIRST CHALLENGE

| Animal number | DAY 24 2%# | DAY 24 Vehicle* | DAY 25 2%# | DAY 25 Vehicle* | Comments |
|---|---|---|---|---|---|
| Control | | | | | |
| 831 | 0 | 0 | 0 | 0 | |
| 832 | 0 | 0 | 0 | 0 | |
| 833 | 0 | 0 | 0 | 0 | |
| 834 | 0 | 0 | 0 | 0 | |
| 835 | 0 | 0 | 0 | 0 | |
| Experimental | | | | | |
| 836 | 0 | 0 | 0 | 0 | |
| 837 | 0 | 0 | 0 p | 0 | |
| 838 | 0 | 0 | 0 p | 0 | |
| 839 | 0 | 0 | 0 | 0 | |
| 840 | 0 | 0 | 0 | 0 | |
| 841 | 0 | 0 | 1 p | 0 | |
| 842 | 1 | 0 | 0 p | 0 | |
| 843 | 0 | 0 | 0 p | 0 | |
| 844 | 0 | 0 | 1 p | 0 | |
| 845 | 0 | 0 | 0 | 0 | |

#. Test substance concentration.

*. Corn oil

p. Scaliness

TABLE 4: CHALLENGE READINGS, SECOND CHALLENGE

| Animal number | DAY 31 2%# | DAY 31 Vehicle* | DAY 32 2%# | DAY 32 Vehicle* | Comments |
|---|---|---|---|---|---|
| Control | | | | | |
| 831 | 0 | 0 | 0 | 0 | |
| 832 | 0 | 0 | 0 | 0 | |
| 833 | 0 | 0 | 0 | 0 | |
| 834 | 0 | 0 | 0 | 0 | |
| 835 | 0 | 0 | 0 | 0 | |
| Experimental | | | | | |
| 836 | 0 | 0 | 0 | 0 | not sensitised |
| 837 | 1 | 0 | 2 p | 0 | sensitised |
| 838 | 0 | 0 | 1 p | 0 | sensitised |
| 839 | 0 | 0 | 0 p | 0 | not sensitised |
| 840 | 0 | 0 | 0 | 0 | not sensitised |
| 841 | 1 | 0 | 1 p | 0 | sensitised |
| 842 | 0 | 0 | 1 p | 0 | sensitised |
| 843 | 1 | 0 | 1 p | 0 | sensitised |
| 844 | 0 | 0 | 1 | 0 | sensitised |
| 845 | 1 | 0 | 1 p | 0 | not sensitised |

#. Test substance concentration.

*. Corn oil

p. Scaliness

TABLE 5 BODY WEIGHTS (GRAM)

| SEX/DOSE LEVEL | ANIMAL | DAY 1 | DAY 32 |
|---|---|---|---|
| **FEMALES CONTROL** | | | |
| | 831 | 395 | 567 |
| | 832 | 360 | 471 |
| | 833 | 365 | 545 |
| | 834 | 347 | 475 |
| | 835 | 369 | 537 |
| | MEAN | 367 | 519 |
| | ST.DEV. | 18 | 43 |
| | N | 5 | 5 |
| **FEMALES EXPERIMENTAL** | | | |
| | 836 | 359 | 531 |
| | 837 | 344 | 549 |
| | 838 | 339 | 479 |
| | 839 | 353 | 522 |
| | 840 | 367 | 524 |
| | 841 | 381 | 543 |
| | 842 | 316 | 414 |
| | 843 | 357 | 512 |
| | 844 | 338 | 488 |
| | 845 | 343 | 509 |
| | MEAN | 350 | 507 |
| | ST.DEV. | 18 | 39 |
| | N | 10 | 10 |

# APPENDIX

## ASSESSMENT OF CONTACT HYPERSENSITIVITY TO ALPHA-HEXYLCINNAMIC ALDEHYDE, TECH. 85% IN THE ALBINO GUINEA PIG (MAXIMISATION-TEST), a Reliability Check.

**Species, Guinea pig, Himalayan strain.**

**NOTOX Project 337376**

## SUMMARY

A reliability check is carried out at regular intervals to check the sensitivity of the test system and the reliability of the experimental techniques as used by NOTOX. In this study, performed in October/November 2001, females of the albino Himalayan guinea pig (from Biotechnology & Animal Breeding Division (RCC Ltd.), Füllinsdorf, Switzerland) were checked for the sensitivity to ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85%. The females were approx. 4 weeks old at commencement of the study. The study was based on the OECD Guideline No. 406, the EC Directive 96/54/EC, Part B.6 and on the method described in "Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens" Magnusson and Kligman, 1970. ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85% (CAS no. 101-86-0) was fabricated under lot no. 10021HF (Aldrich Chemicals Co., Germany).

Test substance concentrations selected for this study were:
Intradermal induction: A 5% solution in water (Milli-U, w/w).
Epidermal induction: undiluted.
First challenge: a 10% solution in water (w/w).
Second challenge: a 20 and 50% solution in water (w/w).

SKIN REACTIONS IN THE CHALLENGE PHASE (Number of animals with skin reactions)

| | Vehicle water (Milli-U) 24/48* | ALPHA-HEXYLCINNAMICALDEHYDE 10% 24/48* | 20% 24/48* | 50% 24/48* |
|---|---|---|---|---|
| Experimental group, 10 females | | | | |
| Score 2 | 0/0 | 0/1 | 0/0 | 1/0a |
| Score 1 | 0/0 | 0/1 | 3/1 | 7/3a |
| No reactions | 10/10 | 10/8 | 7/9a | 2/7a |
| Control group, 5 females | | | | |
| Score 2 | 0/0 | 0/0 | 0/0 | 0/0 |
| Score 1 | 0/0 | 0/0 | 0/0 | 0/0 |
| No reactions | 5/5 | 5/5 | 5/5 | 5/5 |

*. time (hours) after the challenge exposure.
a. some animals also showed scaliness.

## CONCLUSION

The skin reactions in all experimental animals observed in response to the 50% test substance concentration in the challenge phase were considered indicative of sensitisation, based on the absence of any response in the control animals. These results lead to a sensitisation rate of 80 per cent to the 50% concentration. From these results, it was concluded that the female guinea pig of the albino Himalayan strain is an appropriate animal model for the performance of studies designed to evaluate the sensitising potential of a substance in a Maximisation type of test.

The raw data, protocol and report from this study are kept in the NOTOX archives. The test described above was performed in accordance with NOTOX Standard Operating Procedures and the report was audited by the QA-unit.

[redacted]

# *Certificate of Analysis*

[redacted]

TNA-2001007
page 1 of 2

ICS-331

**Product name** : [redacted]

**Chemical name** : [redacted]

**Batch number** : **1510-14**

**Test results**:

| Method | Analysis of | Unit | Result *1 |
|---|---|---|---|
| Jo/72.11, Jo/95.2 | Peroxidic compounds (sum) ***See page 2 for a specification*** | % m/m | 28.6 (± 1.5) |
| J20010792 | [redacted] | % m/m | 67.0 (± 1.0) |
| J20010792 | [redacted] | % m/m | 2.0 (± 0.3) |
| Amp/88.9 | Water | % m/m | 2.6 (± 0.3) |
| J20010792 | Unidentified impurities | % m/m | 0.5 (± 0.2) |

*1 bracketed values are estimated 95% confidence intervals

File code : **TNA-2001007**

Analytical documentation : 20010792

[redacted]

[redacted]

# *Certificate of Analysis*



[redacted]batch 1510-14: specification of the peroxidic compounds

| structure | % m/m |
|---|---|
| [redacted] | |

[redacted]